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Preliminary and Short Report

FLUOROMETRIC DETERMINATION OF QUININE AND FLUORESCEIN EXCRETION IN HUMAN SWEAT*

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Physiologists have long been interested in the chemical composition of sweat (1, 2). From their studies has evolved our concept of sweat as the resultant of two distinct processes, *viz.* tubular secretion and ductal reabsorption. By contrast, clinical knowledge of sweat chemistry has lagged. Only recently have studies on the sweat of cystic fibrosis patients (3) brought the gland into diagnostic prominence.

It has been our feeling that clinical assessment of the sweat gland could be facilitated if one had a "tracer" drug substance which was secreted by the gland and which could easily be measured chemically. One can quickly encompass the background literature on the excretion of drugs by the sweat gland. Tachau (4) was one of the first to review this field. Using quantities of sweat as large as 1500 ml, he was able to demonstrate the secretion of boric acid, bromides, antipyrine and salicylic acid. Koch (5), Cornbleet (6) and others made further observations. In 1954, Weinig and Jahn summarized the findings to date (7) (Table I), and pointed out the possible value of such determinations in medico-legal problems.

The modern development of rapid ultra-sensitive fluorescent assay technics (8) was the stimulus for the present study. It was our hope to find a simple safe drug which could be given to patients and which could be measured easily in small quantities of sweat. As is shown below, quinine proved to be such a sweat tracer element.

METHOD

I. General

All subjects were healthy adult white or Negro males. Sweat was collected from the skin of the upper back after thorough cleansing with fluorometric grade ethyl ether. Drugs were given at the same time each day to ensure regular absorption.

The Turner Fluorometer, Model 111, was used with automatic dial and micro-attachment door (sample, 0.25 ml), (G. K. Turner Associates, Palo Alto, California). Glassware was Pyrex throughout. Reagents were of fluorometric grade (Hartman-Leddon Company, Philadelphia).

Sweating was induced in a sweat-chamber (110° F, relative humidity 80%). Under these conditions, profuse sweating occurred within 10 minutes. Sweat was collected with capillary tubes 75 mm x 1.3–1.5 mm. Scraping of the skin with the tubes was avoided, only distinct drops of sweat being aspirated. Approximately 0.25–0.5 ml sweat were collected within 5 minutes, and immediately sealed with Parafilm® and kept at 0° C until assayed several hours later. The specimens were spun at 20,000 RPM in a micro-centrifuge and the clear cell free supernatant analyzed.

II. Fluorescent Assays

A. *Quinine*. The procedure used is specific for quinine even in the presence of its known metabolites (9). The primary fluorometer filter was Turner №7-60, and the secondary filter №48. Readings were made at the 1× range aperture. The results are shown in Table II. It can be seen that there is a distribution with positive skewness. Quinine was detected in the sweat of all subjects. A number of subjects were further studied at 24 hours. In all cases quinine continued to be present in the sweat at measurable levels.

B. *Fluorescein*. Three hundred μL of sweat were vortexed with 30 μL 1 N Na OH, centrifuged at 20,000 RPM for 3 minutes and read in the Turner Fluorometer using the 10× range aperture. The primary filter was a combination of №47B and №2A, and the secondary filter was №2A-12.

C. *Chlortetracycline*. Two hundred and fifty μL of sweat were vortexed with 60 μL of 1 N Na OH. Readings were made at 60 minutes (primary filter №7-60; secondary filter combination №47B and №2A).

D. *Acetyl salicylic acid* (reference 8, page 424).

E. *Griseofulvin* (reference 8, page 412).

F. *Riboflavin* (reference 8, page 243).

DISCUSSION

The present study is the first to demonstrate quinine excretion in sweat. This compound regularly appeared in significant quantities following an oral dose of 600 mgm. The technic employed by us permitted the use of a sample of 0.25 ml Hence quinine would appear to be a suitable tracer for clearance studies of the sweat gland in health and disease.

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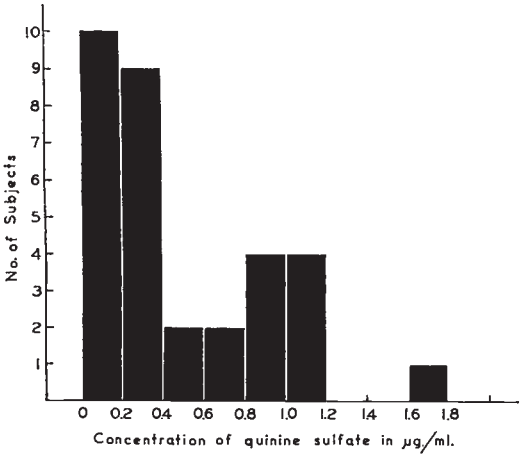
TABLE I

Summary of drugs previously found in sweat (Weinig and Jahn, 1954, ref. 7)

Alcohol	Methylene blue
Antipyrine	Penicillin
Arsenic	Phenol
Barbiturates	Salicylic acid
Boric acid	Salol
Bromides	Sulfonamides
Iodides	Thiocyanate
Mercury	Urotropin

TABLE II

Quinine concentration in human sweat two hours after oral administration of 600 mg quinine sulfate in thirty-two subjects



Fluorescein is a second compound which we have been able to detect and measure fluorometrically in sweat. It has the advantage of being given intravenously so that the variable factors of absorption through the gut are eliminated. Interestingly, Hurley and Witkowski (10) had qualitatively demonstrated the excretion of fluorescein in human sweat. The present technic permits quantitative assay of this compound and hence assessment of its passage through the sweat gland.

Finally, it should be pointed out that several other fluorescent compounds which were surveyed by us failed to appear in sweat in measurable quantities. Of particular note is the fact that riboflavin is in this group. This is in agreement with earlier studies of Sargent, Robinson and Johnson (11) who reported that riboflavin is not lost in sweat.

SUMMARY

The secretion of quinine and fluorescein by the human sweat gland has been measured fluorometrically. Acetyl salicylic acid, chlortetracycline, griseofulvin and riboflavin could not be detected in sweat.

Quinine and fluorescein have been proposed as tracer compounds for further studies of the secretory function of the sweat gland in health and disease.

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TABLE III

Excretion of drugs in human sweat

Drug	No. of Subject	Dose	Collection Time	Concentration (Mean)
Quinine	32	600 mgm (oral)	2 hour	See Figure
Fluorescein	3	500 mgm (I. V.)	0	Not detected
			¼ hour	0.137 µg/ml
			1 hour	0.049 µg/ml
			2 hour	0.016 µg/ml
			3 hour	0.021 µg/ml
			24 hour	0.012 µg/ml
Aspirin	6	1300 mgm (oral)	2 hour	not detected
Chlortetracycline	4	1000 " "	2 hour	not detected (less than 0.1 µg/ml)
Griseofulvin	6	1000 " "	3 hour	not detected
Riboflavin	5	100 " "	2 hour	not detected (less than 0.2 µg/ml)

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